

## Total Exosome Isolation (from cell culture media)

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Store at 2°C to 8°C

### Product Description

Exosomes are small vesicles (30–120 nm) containing RNA and protein that are secreted by various types of cells in culture, and found in abundance in body fluids including blood, saliva, urine, and breast milk. Exosomes are thought to function as intercellular messengers, delivering their cargo of effector or signaling macromolecules between specific cells, however, their formation, the makeup of the cargo, and biological pathways in which they are involved remain incompletely understood.

The biological study of exosome function and trafficking requires the isolation of intact exosomes, but the current methods used are tedious, non-specific, and difficult. The Total Exosome Isolation (from cell culture media) reagent provides a simple and reliable method of concentrating intact exosomes from cell culture media samples. By tying up water molecules, the Total Exosome Isolation (from cell culture media) reagent forces less-soluble components (i.e. exosomes) out of solution, allowing them to be collected after brief, low-speed centrifugation.

### Product Contents

Total Exosome Isolation (from cell culture media) reagent contains reagents sufficient for processing 100 mL of cell culture media.

Components	Amount	Storage
Total Exosome Isolation (from cell culture media)	50 mL	2°C to 8°C

### General Guidelines

- To ensure that isolated exosomes originate from your cells of interest, culture the cells with exosome depleted fetal bovine serum (FBS), because normal FBS contains extremely high levels of exosomes that will contaminate the cell derived exosomes.  
If you cannot obtain exosome depleted FBS, certain cell lines can be grown for up to 12 hours in media without FBS.
- If you are isolating intact exosomes from serum, use the Total Exosome Isolation (from serum) reagent.
- After exosomes are isolated, total RNA and protein can be purified using the Total Exosome RNA and Protein Isolation Kit.
- To isolate exosomal proteins for immunoprecipitation, use Exosome Immunoprecipitation (Protein A) or Exosome Immunoprecipitation (Protein G).

### Prepare Sample

- Harvest cell culture media.
- Centrifuge the cell media at 2000 × g for 30 minutes to remove cells and debris.
- Transfer the supernatant containing the cell-free culture media to a new tube without disturbing the pellet.

### Isolate Exosomes

- Transfer the required volume of cell-free culture media to a new tube and add 0.5 volumes of the Total Exosome Isolation (from cell culture media) reagent.

Culture Media	Reagent
1 mL	500 µL
10 mL	5 mL

- Mix the culture media/reagent mixture well by vortexing, or pipetting up and down until there is a homogenous solution.
- Incubate samples at 2°C to 8°C overnight.
- After incubation, centrifuge the samples at 10,000 × g for 1 hour at 2°C to 8°C.
- Aspirate and discard the supernatant. Exosomes are contained in the pellet at the bottom of the tube (not visible in most cases).
- Resuspend the pellet in a convenient volume of 1X PBS or similar buffer.

Starting Cell Culture Media Volume	Resuspension Volume
1 mL	25–100 µL
10 mL	100 µL–1 mL

- Once the pellet is resuspended, the exosomes are ready for downstream analysis or further purification through affinity methods.  
Keep isolated exosomes at 2°C to 8°C for up to 1 week, or at ≤20°C for long-term storage.

## Related Products

Product	Cat. No.
Total Exosome RNA and Protein Isolation Kit	4478545
Total Exosome Isolation (from serum)	4478360
Exosome Immunoprecipitation (Protein A)	10610D
Exosome Immunoprecipitation (Protein G )	10612D

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